BETA-GALACTOSIDASE ENZYME ASSAY SYSTEM

This protocol is adapted from Promega's Beta-Galactosidase Enzyme Assay System Protocol by the Gene Expression Lab.
This protocol is for use with Promega's Beta-Galactosidase Enzyme Assay Systems. For additional technical inquiries, contact Technical Service at 800-356-9526 or www.promega.com

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BEFORE STARTING THE EXPERIMENT
β-GALACTOSIDASE ASSAY
STANDARD CURVE

BEFORE STARTING THE EXPERIMENT

- Cells are collected from transfection plates following protocol in Transient Co-Transfection Protocol
- Thaw 2X Buffer
- Prepare 1X RLB (Reporter Lysis Buffer)
    - Add 4 volumes of water to 1 volume of 5X RLB to produce a 1X stock solution.

β-Galactosidase Assay

1. Place the Assay 2X Buffer on ice.
2. It will be necessary to dilute the cell lysates in 1X Reporter Lysis Buffer. A 2:1 dilution of lysate to 1X Reporter Lysis Buffer (100µl of lysate plus 50µl of 1X Reporter Lysis Buffer) is a good starting dilution, but up to 150µl of cell lysate can be used per reaction. As a negative control, prepare the same dilution of a cell lysate made from cells that have not been transfected with the β-galactosidase gene.
3. Pipet 150µl of the appropriately diluted (or undiluted) cell lysates into labeled tubes.
4. Add 300 µL of DEPC H_20 into each tube (to bring the total volume for each tube of the assay to 1.1 mL for spectrophotometer requirements)
5. Add 150 µL of Assay 2X Buffer to each of the tubes & Mix all samples by vortexing briefly.
6. Incubate the reactions at 37°C for 60 minutes or until a faint yellow color develops. Color development continues for approximately 3 hours. If enzyme activity is low, samples may be incubated overnight if the reaction tubes are tightly capped.

7. Stop the reactions by adding 500 µL of 1M Sodium Carbonate (1x RLB used to lyse cells) or 500 µL of 1m Tris Base (1x RLA used to lyse cells). Mix by vortexing briefly.
8. Read the absorbance at 420nm.

Standard Curves

1. If a standard curve is desired, use standards between 0 and 6.0 x 10–3 units of β-Galactosidase. Prepare the following dilution series in 1X Reporter Lysis Buffer immediately before use.
2. Add 10 µL of 1u/µL β-Galactosidase to 990 µL of 1X Reporter Lysis Buffer and mix.
3. Then add 10 µL of this 1:100 dilution to 990 µL of 1X Reporter Lysis Buffer and mix it to make a 1:10,000 stock solution.
4. Using this stock, prepare 150 µL of each β-Galactosidase standard per tube as described below.

β -Galactosidase Standard (milliunits)	Volume of 1:10,000 Stock	Volume of 1X Reporter Lysis Buffer
0	0µl	50µl
1.0	10µl	40µl
2.0	20µl	30µl
3.0	30µl	20µl
4.0	40µl	10µl
5.0	50µl	0µl

5. Follow the protocol described in Steps 4–8 in the β-Galactosidase Assay section above.
6. Plot the absorbance at 420nm versus concentration of β-Galactosidase standards.
7. If a standard curve is used, prepare fresh enzyme dilutions.